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(54) Title: MU-1, MEMBER OF THE CYTOKINE RECEPTOR FAMILY

(57) Abstract: Polynucleotides encoding the MU-1 hematopoietin receptor superfamily chain and fragments thereof are disclosed. MU-1 proteins and methods for their production are also disclosed.



WO 01/85792 A2

MU-1, MEMBER OF THE CYTOKINE RECEPTOR FAMILY

Field of the Invention

The present invention relates to new members of the mammalian cytokine
5 receptor family of proteins (including without limitation human and murine receptor
proteins), fragments thereof and recombinant polynucleotides and cells useful for
expressing such proteins.

Background of the Invention

10 A variety of regulatory molecules, known as hematopoietins, have been
identified which are involved in the development and proliferation of the various
populations of hematopoietic or blood cells. Most hematopoietins exhibits certain
biological activities by interacting with a receptor on the surface of target cells.
Cytokine receptors are commonly composed of one, two or three chains. Many cytokine
15 receptors and some cytokines, such as IL-12 p40, are members of the hematopoietin
receptor superfamily of proteins. Identification of new members of the hematopoietin
receptor superfamily can be useful in regulation of hematopoiesis, in regulation of
immune responses and in identification of other members of the hematopoietin
superfamily, including cytokines and receptors.

20 It would be desirable to identify and determine the DNA and protein sequence
for heretofore unknown members of the hematopoietin receptor superfamily.

Summary of the Invention

In accordance with the present invention, polynucleotides encoding the MU-1
25 hematopoietin receptor superfamily chain are disclosed, including without limitation
those from the murine and human sources.

In certain embodiments, the invention provides an isolated polynucleotide
comprising a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence of SEQ ID NO:1;
- 30 (b) the nucleotide sequence of SEQ ID NO:1 from nucleotide 238 to
nucleotide 1852;

(c) the nucleotide sequence of SEQ ID NO:1 from nucleotide 301 to nucleotide 1852;

(d) the nucleotide sequence of SEQ ID NO:1 from nucleotide 301 to nucleotide 945;

5 (e) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in any of (a)-(d) as a result of degeneracy of the genetic code;

(f) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in any of (a)-(d);

(g) a nucleotide sequence encoding a species homologue of the sequence of
10 SEQ ID NO:2; and

(h) an allelic variant of the nucleotide sequence specified in any of (a)-(d). Preferably, the nucleotide sequence encodes a protein having a biological activity of the MU-1 hematopoietin receptor superfamily chain. The nucleotide sequence may be operably linked to an expression control sequence.

15 The invention also provides isolated polynucleotides comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 538;

20 (c) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 236;

(d) the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 236; and

(e) fragments of (a)-(d) having a biological activity of the MU-1 hematopoietin receptor superfamily chain.

25 In another embodiment, the invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:9;

(b) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code;

30 (c) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a);

- (d) an allelic variant of the nucleotide sequence specified in (a).

The invention also provides isolated polynucleotides comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10;
(b) fragments of (a) having a biological activity of the MU-1 hematopoietin receptor superfamily chain.

Host cells, preferably mammalian cells, transformed with the polynucleotides are also provided.

In other embodiments, the invention provides a process for producing a MU-1 protein. The process comprises:

- (a) growing a culture of the host cell of the present invention in a suitable culture medium; and

- (b) purifying the human MU-1 protein from the culture.

Proteins produced according to these methods are also provided.

The present invention also provides for an isolated MU-1 protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
(b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 538;
(c) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 236;
(d) the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 236; and
(e) fragments of (a)-(d) having a biological activity of the MU-1 hematopoietin receptor superfamily chain.

The present invention also provides for an isolated MU-1 protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10;
(b) fragments of (a) having a biological activity of the MU-1 hematopoietin receptor superfamily chain.

The amino acid sequence of Murine MU-1 (SEQ ID NO:10) is about 65% identical to the amino acid sequence of human MU-1.

In other preferred embodiments, the specified amino acid sequence is part of a fusion protein (with an additional amino acid sequence not derived from MU-1).

5 Preferred fusion proteins comprise an antibody fragment, such as an Fc fragment.

Pharmaceutical compositions comprising a protein of the present invention and a pharmaceutically acceptable carrier are also provided.

The present invention further provides for compositions comprising an antibody which specifically reacts with a protein of the present invention.

10 In one embodiment, a MU-1 nucleic acid molecule of the invention is at least 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1 or 9.

In another preferred embodiment, the nucleic acid molecule consists of the
15 nucleotide sequence shown in SEQ ID NO:1 or 9. In another preferred embodiment, the nucleic acid molecule includes a fragment of at least 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, or more nucleotides (*e.g.*, contiguous nucleotides) of the nucleotide sequence of SEQ ID NO:1 or 9, or a complement thereof.

20 In a preferred embodiment, the MU-1 protein family member has an amino acid sequence at least about 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identical to the amino acid sequence of SEQ ID NO:2 or 10.

In another embodiment, the invention features fragments of the protein having
25 the amino acid sequence of SEQ ID NO:2 or 10, wherein the fragment comprises at least 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids (*e.g.*, contiguous amino acids) of the amino acid sequence of SEQ ID NO:2 or 10.

In another aspect, the present invention provides a method for detecting the presence of a MU-1 nucleic acid molecule, protein or polypeptide in a biological sample
30 by contacting the biological sample with an agent capable of detecting a MU-1 nucleic

acid molecule, protein or polypeptide such that the presence of a MU-1 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of MU-1 activity in a biological sample by contacting the biological sample
5 with an agent capable of detecting an indicator of MU-1 activity such that the presence of MU-1 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating MU-1 activity comprising contacting a cell capable of expressing MU-1 with an agent that modulates MU-1 activity such that MU-1 activity in the cell is modulated. In one embodiment, the
10 agent inhibits MU-1 activity. In another embodiment, the agent stimulates MU-1 activity. In one embodiment, the agent is an antibody that specifically binds to a MU-1 protein. In another embodiment, the agent modulates expression of MU-1 by modulating transcription of a MU-1 gene or translation of a MU-1 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence
15 that is antisense to the coding strand of a MU-1 mRNA or a MU-1 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant or unwanted MU-1 protein or nucleic acid expression or activity (*e.g.*, an MU-1 associated disorder) by administering an agent which is a MU-1 modulator to the subject. In one embodiment, the MU-1 modulator is
20 an MU-1 protein. In another embodiment the MU-1 modulator is an MU-1 nucleic acid molecule. In yet another embodiment, the MU-1 modulator is a peptide, peptidomimetic, antibody, or other small molecule.

The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant
25 modification or mutation of a gene encoding a MU-1 protein; (ii) mis-regulation of the MU-1 gene; and (iii) aberrant post-translational modification of an MU-1 protein, wherein a wild-type form of the gene encodes a protein with a MU-1 activity.

In another aspect the invention provides methods for identifying a compound that binds to or modulates the activity of a MU -1 protein, by providing an indicator
30 composition comprising a MU-1 protein having MU-1 activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on

MU-1 activity (e.g., modulation of STAT phosphorylation, e.g., STAT 3 or STAT 5 phosphorylation) in the indicator composition to identify a compound that modulates the activity of a MU-1 protein.

5

Brief Description of the Drawings

Figure 1 depicts the full-length cDNA sequence of murine MU-1. The nucleotide sequence corresponds to nucleotides 1-2628 of SEQ ID NO:9.

Figure 2 depicts the amino acid sequence of murine MU-1 (corresponding to the amino acids 1-529 of SEQ ID NO:10). There is a predicted leader sequence at amino acids 1-19, which was predicted by SPScan with score of 10.1 (bold-face type). There is a predicted transmembrane domain at amino acids 237-253 (underlined). Predicted signaling motifs include the following regions: Box 1: amino acids 265-274 and Box 2: amino acids 310-324 (bold and underlined); Six Tyrosines are located at amino acid positions 281, 319, 361, 368, 397, and 510. The WSXWS motif (SEQ ID NO:8) is located at amino acid residue 214 to amino acid residue 218 (in large, bold-face type). Potential STAT docking sites include amino acids 393-398 and amino acids 510-513.

Figure 3 depicts the GAP comparison of human and murine MU-1 cDNA sequences (corresponding to nucleic acids 1-2665 of SEQ ID NO:1 and nucleic acids 1-2628 of SEQ ID NO:9, respectively). HuMU-1= human MU-1, murMU-1= murine MU-1. Gap Parameters: Gap Weight= 50, Average Match= 10.000, Length Weight= 3, Average Mismatch= 0.000. Percent Identity= 66.116.

Figure 4 depicts a GAP comparison of the human MU-1 protein (corresponding to amino acids 1-538 of SEQ ID NO:2) and the murine MU-1 protein (corresponding to amino acids 1-529 of SEQ ID NO:10). BLOSUM62 amino acid substitution matrix. (Henikoff, S. and Henikoff, J. G. (1992)). Amino acid substitution matrices from protein blocks (Proc. Natl. Acad. Sci. USA 89: 10915-10919). Gap parameters= Gap Weight: 8, Average Match= 2.912, Length Weight= 2, Average Mismatch= -2.003. Percent Identity= 65.267

Figure 5 depicts a multiple sequence alignment of the amino acids of human MU-1 (corresponding to SEQ ID NO:2), murine MU-1 (corresponding to SEQ ID

NO:10), and human IL2beta chain (GENbank Accession No. M26062). Leader and transmembrane domains are underlined. Conserved cytokine receptor module motifs are indicated by bold-face type. Potential signaling regions are indicated by underlining and bold-face type.

- 5 *Figure 6* depicts signaling through MU-1. MU-1 phosphorylates STAT 5 in Clone E7 EPO-MU-1 chimera. Under the conditions specified in Example 3, signaling through MU-1 results in the phosphorylation of STAT 5 at all time-points tested. Treatment of controls or the chimeric BAF-3 cells with IL-3 resulted in phosphorylation of STAT 3, but not STAT 1 or 5.

10

Detailed Description of Preferred Embodiment

The inventors of the present application have for the first time identified and provided polynucleotides encoding the MU-1 hematopoietin receptor superfamily chain (hereinafter "MU-1" or "MU-1 protein"), including without limitation polynucleotides
15 encoding human and murine MU-1.

In a particularly preferred embodiment, the MU-1 protein and nucleic acid molecules of the present invention are human MU-1 molecules. A 70 amino acid region of the human IL5 receptor

(LMTNAFISIIDDLSKYDVQVRAAVSSMCREAGLWSEWSQPIYVGND

- 20 EHKPLREWFVIVIMATICFILLIL, SEQ ID NO:3) was used to search the GenBank EST database using the TBLASTN algorithm. Sequence within the genomic BAC clone AC002303 from human chromosome 16p12 was identified with homology to this region, suggesting that this segment might encode a gene for a novel hematopoietin receptor. Examination of open reading frames within 1000bp of nucleotide 40,886
25 revealed a 270bp open frame which when used in a BLASTP search of GenPept exclusively identified members of the cytokine receptor family. A stop codon present at the end of this reading frame was interpreted as indication of transition over an exon/intron border.

- It was then determined whether RNA was transcribed from a gene contained
30 within this BAC clone from chromosome 16p12. PCR primers were synthesized based on the largest ORF segment which contained peptide sequence conserved within the

cytokine receptor family. Primers GAGTCCGAGGAGAAAGCTGATCTCA (5p) (SEQ ID NO:4) and GAAAGATGACCGGGTCACTCCATT (3p) (SEQ ID NO:5) were used in PCRs to screen phage libraries from various human tissues (Clontech). PCR products of the expected 164 bp size which specifically hybridized to a 32-P labeled oligonucleotide of the sequence ACTCGAGCTATGAGCTGCAGGTGCGGGCA (SEQ ID NO:6) were observed in phage from lung, kidney, placenta and heart. Using the oligonucleotide ACTCGAGCTATGAGCTGCAGGTGCGGGCA (SEQ ID NO:7) a full-length cDNA clone NN14-1b (MU-1) was identified, purified, and sequenced. The DNA sequence and the predicted amino acid sequence are shown in SEQ ID NO:1 and SEQ ID NO:2, respectively. The predicted amino acid sequence of the human MU-1 receptor chain includes a putative signal sequence from amino acids 1-21. The mature human MU-1 is believed to have the sequence of amino acids 24-538 of SEQ ID NO:2. A transmembrane domain is found at amino acids 237-254.

In another particularly preferred embodiment, the MU-1 protein and nucleic acid molecules of the present invention are murine MU-1 molecules. To identify the polynucleotide sequence encoding the murine MU-1 protein a partial fragment of the murine homolog of the MU-1 receptor was isolated by PCR from mouse cDNA using oligonucleotides derived from the human sequence, as described in Example 1. The DNA sequence of this fragment was determined, and two oligonucleotides were derived from an internal portion of this fragment with the following sequences:

TTGAACGTGACTGTGGCCTT (5p) (SEQ ID NO:13)

TGAATGAAGTGCCTGGCTGA (3p) (SEQ ID NO:14).

The oligonucleotides were used to amplify an internal 262 nucleotide fragment of the original PCR product (corresponding to nucleotides 781-1043 of the murine cDNA sequence of figure 1 and SEQ ID NO:9) to use as a hybridization probe to screen a cDNA library isolated from the 2D6 T cell line. DNA sequence was determined from two independent clones. Clone 6 was sequenced and confirmed to be the full-length murine homolog of human MU-1.

The full-length nucleotide sequence of murine MU-1 is shown in figure 1 (corresponding to nucleotides 1-2628 of SEQ ID NO:9). The nucleotide sequence has a predicted leader sequence at nucleotides 407-464, coding sequence at nucleotides 407-1993, and a termination codon at nucleotides 1994-1997. Nucleotides 1-406 correspond to the 5' untranslated region and nucleotides 1998-2628 correspond to the 3' untranslated region. The predicted protein sequence of murine MU-1 is shown in figure 2 (corresponding to amino acids 1-529 of SEQ ID NO:10).

The murine MU-1 protein contains a predicted leader sequence determined by SPScan (score=10.1) (corresponding to amino acids 1-19 of SEQ ID NO:10), and a predicted transmembrane domain (corresponding to amino acids 237-253 of SEQ ID NO:10). Predicted signaling motifs include the following regions: Box 1: amino acids 265-274 of SEQ ID NO:10, Box 2: amino acids 310-324 of SEQ ID NO:10, six tyrosine residues at positions 281, 319, 361, 368, 397, and 510 of SEQ ID NO:10. Potential STAT docking sites include but are not limited to: STAT 5: EDDGYPA, STAT 3:YLQR.

The open reading frame of MU-1 encodes a novel member of the hematopoietin receptor family. In a preferred embodiment, MU-1 has a leader sequence, conserved cysteine pairs, PP, and WSXWS (SEQ ID NO:8) motifs characteristic of the family, as well as a transmembrane domain and an extensive cytoplasmic domain. MU-1 also contains a conserved PXPP as well as Box I and Box II signaling motifs in the cytoplasmic domain (see Figure 5). These domains are conserved between murine MU-1 and human MU-1. Subsequent FASTA alignment of MU-1 sequence with GenPept showed greatest homology with human IL-2Rb (see figure 5).

The human MU-1 cDNA was deposited with the American Type Culture Collection on March 10, 1998, as accession number ATCC 98687.

By Northern analysis, as described in Example 4, murine MU-1 was detected in adult murine spleen, lung, and heart tissues. Human MU-1 was detected in adult human lymphoid tissues, PBLs, thymus, spleen and lymph node, and in fetal lung.

Any forms of MU-1 proteins of less than full length are encompassed within the present invention and are referred to herein collectively with full length and mature forms as "MU-1" or "MU-1 proteins." MU-1 proteins of less than full length may be

produced by expressing a corresponding fragment of the polynucleotide encoding the full-length MU-1 protein (SEQ ID NO:4 or SEQ ID NO:6). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis methods or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "a biological activity of the MU-1 hematopoietin receptor superfamily chain" if it possess one or more of the biological activities of the corresponding mature MU-1 protein. In one embodiment, MU-1 activity includes interaction with STAT molecules (e.g., STAT 5, STAT 3). In another embodiment, the MU-1 protein activity does not include binding to any known cytokines.

MU-1 or active fragments thereof (MU-1 proteins) may be fused to carrier molecules such as immunoglobulins or immunoglobulin fragment. For example, soluble forms of the MU-1 may be fused through "linker" sequences to the Fc portion of an immunoglobulin. Other fusion proteins, such as those with GST, Lex-A or MBP, may also be used.

The invention also encompasses allelic variants of the nucleotide sequence as set forth in SEQ ID NO:1 and SEQ ID NO:9, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:1 or SEQ ID NO:9 which also encode MU-1 proteins, preferably those proteins having a biological activity of MU-1. Also included in the invention are isolated polynucleotides which hybridize to the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:9 under highly stringent conditions (for example, 0.1xSSC at 65°C). Isolated polynucleotides which encode MU-1 proteins but which differ from the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:9 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:9 which are caused by point mutations or by induced modifications are also included in the invention.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65°C. Ranges that span the above recited values, e.g., 55-60°C, or 50-65°C are encompassed by the present invention. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 or 9 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by

the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred
5 embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet
10 another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the
15 algorithm of E. Meyers and W. Miller (Myers and Miller, 1988, *Comput. Appl. Biosci.* 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example,
20 identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to Adhr-1 nucleic acid molecules of the invention. BLAST protein
25 searches can be performed with the XBLAST program, score = 100, wordlength = 3 to obtain amino acid sequences homologous to Adhr-1 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective
30 programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The present invention also provides polynucleotides encoding homologues of the human MU-1 from other animal species, particularly other mammalian species. Species homologues can be identified and isolated by making probes or primers from the murine or human sequences disclosed herein and screening a library from an appropriate
5 species, such as for example libraries constructed from PBMCs, thymus or testis of the relevant species.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the MU-1
10 protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control
15 sequence, in such a way that the MU-1 protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the MU-1 protein. Any cell type capable of expressing functional MU-1 protein may be
20 used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D,
25 FDCP-1, PC12, M1x or C2C12 cells.

The MU-1 protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form
30 from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas

Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the MU-1 protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the MU-1 protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins.

Expression in bacteria may result in formation of inclusion bodies incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active or more active material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve solubilizing the protein from the inclusion bodies, then denaturing the protein completely using a chaotropic agent. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno, Meth. Enzym., 185:187-195 (1990). EP 0433225 and copending application USSN 08/163,877 describe other appropriate methods.

The MU-1 protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the MU-1 protein.

The MU-1 protein of the invention may be prepared by growing a culture transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the MU-1 protein of the invention can be purified from conditioned media. Membrane-bound forms of MU-1 protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

The MU-1 protein can be purified using methods known to those skilled in the art. For example, the MU-1 protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) or polyethyleimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the MU-1 protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the MU-1 protein. Affinity columns including antibodies to the MU-1 protein can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated MU-1 protein is purified so that it is substantially free of other mammalian proteins.

MU-1 proteins of the invention may also be used to screen for agents which are capable of binding to MU-1. Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the MU-1 protein of the invention. Purified cell based or protein based (cell free) screening assays may be used to identify such agents. For example, MU-1 protein may be immobilized in purified form on a carrier and binding or potential ligands to purified MU-1 protein may be measured.

MU-1 proteins, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to MU-1 or inhibitor and carrier, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15, G-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may also include anti-cytokine antibodies. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated MU-1 protein, or to minimize side effects caused by the isolated MU-1 protein. Conversely, isolated MU-1 protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which isolated MU-1 protein is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of, healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of isolated MU-1 protein is administered to a mammal. Isolated MU-1 protein may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, MU-1 protein may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering MU-1 protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of MU-1 protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of MU-1 protein is administered orally, MU-1 protein will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% MU-1 protein, and preferably from about 25 to 90% MU-1 protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical

composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of MU-1 protein, and preferably from about 1 to 50% MU-1 protein.

When a therapeutically effective amount of MU-1 protein is administered by intravenous, cutaneous or subcutaneous injection, MU-1 protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to MU-1 protein an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of MU-1 protein in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of MU-1 protein with which to treat each individual patient. Initially, the attending physician will administer low doses of MU-1 protein and observe the patient's response. Larger doses of MU-1 protein may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 μ g to about 100 mg of MU-1 protein per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the MU-1 protein will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the

attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

The polynucleotide and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays
5 cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

10 Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations.

Many protein factors discovered to date, including all known cytokines, have
15 exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M⁺ (preB M⁺), 2E8, RB5, DA1, 123,
20 T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

BAF-3 cells expressing huEPOR -huMU chimeric receptors proliferate in response to huEPO. In order to test the capacity for the MU receptor to signal through their cytoplasmic domain, BAF-3 cells were engineered to express EPOr/MU(cyto) chimeric receptors and assayed for ³H thymidine incorporation in the presence of EPO.
25 BAF-3 cells expressing intact EPOr molecules proliferate in response to EPO while parent BAF-3 cells do not. The A5 clone, possessing the chimeric EPOr/MU(cyto) proliferates in response to EPO demonstrating that the cytoplasmic portion of MU-1 can sustain a proliferative signal. The BAF-3 cells which express EPOr on their surface also respond to EPO.

30 The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, *Immunologic studies in Humans*); Takai et al., *J. Immunol.* 137:3494-3500, 1986; Bertagnolli et al., *J. Immunol.* 145:1706-1712, 1990; Bertagnolli et al., *Cellular Immunology* 133:327-341, 1991; Bertagnolli, et al., *J. Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ , Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., *J. Exp. Med.* 173:1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., *Proc. Natl. Acad. Sci. U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H.

5 Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai

10 et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays

15 are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or

20 fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also

25 be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation,

30 Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease.

Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of
5 the present invention.

The MU-1 DNA also maps to the chromosomal locus for Crohn's disease. As a result, proteins of the present invention may be used to treat Crohn's disease and other inflammatory bowel diseases.

Using the proteins of the invention it may also be possible regulate immune
10 responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific,
15 process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the
20 absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For
25 example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such
30 as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen

(e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating

autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and
5 BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing
10 immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

15 Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral
20 immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

25 In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the
30 subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo*

with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy

5 techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class

10 II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface.

15 Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a

20 peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured

25 by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte

30 Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol.

128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., *Cytometry* 13:795-808, 1992; Gorczyca et al., *Leukemia* 7:659-670, 1993;

5 Gorczyca et al., *Cancer Research* 53:1945-1951, 1993; Itoh et al., *Cell* 66:233-243, 1991; Zacharchuk, *Journal of Immunology* 145:4037-4045, 1990; Zamai et al., *Cytometry* 14:891-897, 1993; Gorczyca et al., *International Journal of Oncology* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and

10 development include, without limitation, those described in: Antica et al., *Blood* 84:111-117, 1994; Fine et al., *Cellular Immunology* 155:111-122, 1994; Galy et al., *Blood* 85:2770-2778, 1995; Toki et al., *Proc. Nat. Acad. Sci. USA* 88:7548-7551, 1991.

The MU-1 molecules of the present invention are also involved in the modulation of STAT activity, *e.g.*, signaling (*e.g.*, through interaction with STAT molecules, *e.g.*,

15 STAT 3 and/or STAT 5, and modulation of STAT phosphorylation). Modulators of STAT signaling and modulators of MU-1 interaction with STAT molecules can be identified through screening assays as described herein.

Assays that may be used to identify modulators of MU-1 activity include assays for STAT activity, *e.g.*, STAT signaling, such as assays known in the art and assays

20 described herein. Assays for STAT activity are also described in, for example, Friedrich K, *et al.* (2001) *Biol Chem* 382(2):343-51. Other assays for STAT activity include assays for cell proliferation and STAT phosphorylation.

In one aspect, an assay for a modulator of MU-1 activity is a cell-based assay in which a cell which expresses a MU-1 protein or biologically active portion thereof is

25 contacted with a test compound and the ability of the test compound to modulate MU-1 activity (*e.g.*, interaction with STAT molecules or modulation of STAT signaling) is determined. Determining the ability of the test compound to modulate MU-1 activity can be accomplished by monitoring, for example, STAT signaling, phosphorylation of STAT molecules, cell proliferation, cell differentiation, or production of cytokines. The

30 cell, for example, can be of mammalian origin, *e.g.*, an activated T-cell.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995;

Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Research Uses and Utilities

Polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or

other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a
5 receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to
10 determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a
15 particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can
20 also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in
25 the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

30

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

MU-1 proteins of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the MU-1 protein and which may inhibit binding of ligands to the receptor. Such antibodies may be obtained using the entire MU-1 as an immunogen, or by using fragments of MU-1. Smaller fragments of the MU-1 may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to MU-1 protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies may be capable of blocking ligand binding to the MU-1 receptor chain.

All patent and literature references cited herein are incorporated by reference as if fully set forth. This invention is further illustrated by the following examples which should not be construed as limiting.

EXAMPLES

Example 1: Isolation and Characterization of Murine MU-1 cDNAs:

5 A partial fragment of the murine homolog of the Mu-1 receptor was isolated by PCR using oligonucleotides derived from the human sequence. cDNA was prepared from RNA isolated from 17 day old murine thymus and from the murine 2D6 T cell line. A DNA fragment of approximately 300 nucleotides was amplified from the cDNA by PCR with the following oligonucleotides, corresponding to regions 584-603 and 876-10 896, respectively, of the human cDNA sequence in figure 1 (corresponding to SEQ ID NO:1):

AGCATCAAGC CGGCTCCCCC (5p) (SEQ ID NO:11)

CTCCATTAC TCCAGGTCCC (3p) (SEQ ID NO:12)

15

Amplification was carried out using Taq polymerase in 1 x Taq buffer containing 1.5 mM of magnesium chloride for 30 cycles at 94° C for one minute, 50° C for 1 minute, and 72° C for one minute. The DNA sequence of this fragment was determined, and two oligonucleotides were derived from an internal portion of this fragment with the 20 following sequences:

TTGAACGTGACTGTGGCCTT (5p) (SEQ ID NO:13)

TGAATGAAGTGCCTGGCTGA (3p) (SEQ ID NO:14)

25 The oligonucleotides were used to amplify an internal 262 nucleotide fragment of the original PCR product (corresponding to nucleotides 781-1043 in of the murine cDNA sequence of figure 1, and SEQ ID NO:9) to use as a hybridization probe to screen a cDNA library isolated from the 2D6 T cell line. Filters were hybridized at 65° C using standard 5 X SSC hybridization conditions and washed into SSC at 65° C. Twenty clones 30 were isolated that hybridized to the probe in a screen of 426,000 clones. DNA sequence

was determined from two independent clones. Full length sequence of clone #6 confirmed that it was the full-length murine homolog of human MU-1 (SEQ ID NO:9).

The full-length nucleotide sequence of murine MU-1 is shown in figure 1 (corresponding to SEQ ID NO:9). The nucleotide sequence has a predicted leader
5 sequence at nucleotides 407-464, coding sequence at nucleotides 407-1993, termination codon at nucleotides 1994-1997. Nucleotides 1-406 correspond to the 5' untranslated region and nucleotides 1998-2628 correspond to the 3' untranslated region.

The predicted protein sequence of murine MU-1 is shown in figure 2 (corresponding to SEQ ID NO:10). This murine MU-1 protein contains a predicted
10 leader sequence determined by SPScan (score=10.1) (corresponding to amino acids 1-19 of SEQ ID NO:10), and a predicted transmembrane domain (corresponding to amino acids 237-253 of SEQ ID NO:10). Predicted signaling motifs include the following regions: Box 1: amino acids 265-274 of SEQ ID NO:10, Box 2: amino acids 310-324 of SEQ ID NO:10, six tyrosine residues at positions 281, 319, 361, 368, 397, and 510 of
15 SEQ ID NO:10. Potential STAT docking sites include: STAT5: EDDGYPA, STAT 3:YLQR.

Example 2: Comparison of Human and Murine MU-1:

The GAP algorithm was used to compare the human and murine MU-1 amino
20 acids. A comparison of the murine and human predicted protein sequences is shown in figure 4. The amino acids were 65.267% identical using the GAP algorithm. The alignment was generated by BLOSUM62 amino acid substitution matrix (Henikoff, S. and Henikoff, J. G. (1992)). Amino acid substitution matrices from protein blocks (Proc. Natl. Acad. Sci. USA 89: 10915-10919). Gap parameters= Gap Weight: 8, Average
25 Match= 2.912, Length Weight= 2, Average Mismatch= -2.003. Percent Similarity= 69.466.

A comparison of the human and murine cDNA nucleotide sequences is shown in figure 3. The DNA sequences are 66.116% identical when aligned using the GAP algorithm. Gap Parameters: Gap Weight= 50, Average Match= 10.000, Length Weight=
30 3, Average Mismatch= 0.000. Percent Similarity= 66.198.

Both human and mouse MU-1 proteins are members of the Type 1 cytokine receptor superfamily. Evaluation of the sequence of both murine and human MU-1 reveals the presence of potential Box-1 and Box-2 signaling motifs. Six tyrosine residues are present in the cytoplasmic domain, and could also be important in signaling functions of MU-1. Comparison of the sequences of MU-1 with other members of the family suggested the presence of potential docking sites for STAT 5 and STAT 3.

Example 3: Determination of STAT signaling pathways used by Human MU-1:

BAF-3 cells were engineered to express a chimeric cytokine receptor consisting of the extracellular domain of the human EPO receptor and the intracellular domain of the MU-1 receptor. BAF-3 cells that expressed huEPOR/MU-1(cyto) chimeric receptors proliferated in response to human soluble EPO. These cells were analyzed to determine which STAT molecules were phosphorylated in response to EPO signaling. Briefly, control unmodified parental BAF-3 cells and EPOR/MU chimeric BAF-3 cells were rested from IL-3 containing growth medium, and restimulated with either IL-3 or EPO for 0, 15, 30 and 60 minutes. The cells were pelleted and resuspended in ice cold lysis buffer containing orthovanadate, to preserve phosphorylated tyrosines. Equal amounts of cell lysate were electrophoresed by SDS-PAGE and blotted onto nitrocellulose membranes for western analysis. Duplicate blots were stained for phosphorylated and nonphosphorated forms of STAT 1, 3, 5, and 6 by using antibodies specific for each form of the STAT molecule. HELA cells, non-activated and activated with alpha-interferon were used as positive controls.

These results indicated that under these specific conditions, signaling through MU-1 results in the phosphorylation of STAT 5 at all time-points tested (T=0, T=15', T=30', T=60'). Treatment of controls or the chimeric BAF-3 cells with IL-3 resulted in phosphorylation of STAT 3, but not STAT 1 or 5.

Example 4: Tissue Expression of Murine and Human MU-1

Northern Analysis

Northern blots of polyA+ RNA from various tissues (Clontech, Palo Alto, CA) were performed as recommended by the manufacturer. For the murine blots, a 262 nucleotide fragment corresponding to nucleotides 781-1043 of Fig. 1 and SEQ ID NO:9 was used for hybridization.

A single transcript of murine MU-1 was detected in adult murine spleen, lung, and heart tissues. The larger transcript observed in human tissues was not observed in mouse tissues.

Two transcripts of human MU-1 were detected in adult human lymphoid tissues, PBLs, thymus, spleen and lymph node, and in fetal lung.

In Situ Hybridization

In situ hybridization studies were performed by Phylogency Inc. of Columbus, OH (according to the method of Lyons et.al. , 1990, J. Cell. Biol: 111:2427-2436.) Briefly, serial 5-7 micron paraffin sections were deparaffinized, fixed, digested with proteinase K, treated with tri-ethanolamine and dehydrated. cRNAs were prepared from linearized cDNA templates to generate antisense and sense probes. The cRNA transcripts were synthesized according to manufacturer's conditions (Ambion) and labeled with 35S-UTP. Sections were hybridized overnight, stringently washed and treated with RNAase A and dipped in nuclear track emulsion and exposed for 2-3 weeks. Control sections were hybridized with sense probes to indicate the background level of the procedure. The murine probe consisted of an 186bp fragment corresponding to nucleotides 860-1064 (SEQ ID NO:9). The human probe was a 231bp PCR product generated from human MU-1 DNA.

Murine MU-1 expression was observed in the lymph nodes of the adult small intestine at germinal centers and muscularis externa. Specialized lymph nodes and Peyer's patches also exhibited murine MU-1 expression.

Human MU-1 expression was detected at germinal centers of the lymph nodules in the cortex. The medulla, which contains macrophages, was negative for human MU-1. In human spleen, human MU-1 expression was detected in the regions of white pulp but not red pulp.

Example 5: Expression of Human MU-1 in cells and cell lines:

RNAse protection analysis was performed on resting and activated human T cells and the B cell lines, Raji and RPMI 8866, and the T cell line Jurkat. Human T cells
5 were activated with anti-CD3 and anti-CD28. The cell lines were activated by Phorbol ester and ionomycin. MU-1 riboprobe-producing plasmid was constructed by inserting a 231bp PCR product (PCR was performed by using 5' primer CACAAAGCTTCAGTATGAGCTGCAGTACAGGAACCGGGGA (SEQ ID NO: 15) and 3' primer CACAGGATCCCTTTAACTCCTCTGACTGGGTCTGAAAGAT (SEQ
10 ID NO:16)) into the BamH1 and HindIII sites of pGEM3zf(-) (Promega, Madison, WI) vector. To make the riboprobe, the riboprobe-producing plasmid was linearized with HindIII. The resulting DNA was phenol/chloroform extracted and precipitated with ethanol. T7 RNA polymerase was used to make the riboprobe according to the protocol suggested by the vendor (PharMingen, San Diego, CA). The RNAse protection assay
15 was performed by using PharMingen's RiboQuant Multi-Probe Ribonuclease Protection Assay system. 2.0ug of total RNA were included in each RPA reaction, after RNAse digestion, the protected riboprobes were run on a QuickPoint rapid nucleic acid separation system (Novex, San Diego, CA). Gels were dried and exposed according to the suggestion of the vendor.

20 Human MU-1 RNA is upregulated in anti-CD3 +anti-CD28 stimulated human purified CD3+ cells when compared with unstimulated populations. MU-1 is also upregulated upon restimulation in Th and Th2-skewed T cell populations. The B cell lines, RPMI 8866 and Raji, constitutively express MU-1 while the Jurkat T cell line does not.

25

Example 6: Binding of Human MU-1 to Known Cytokines

Both human and murine Ig fusion proteins were constructed and immobilized on Biacore chips in an effort to identify the ligand for MU-1. A variety of cell culture conditioned media as well as a panel of known cytokines were evaluated for binding to
30 MU-1. Some cytokines were also tested in combination with other receptor chains in the family to consider the possibility that MU-1 may require a second receptor chain for

- ligand binding. The following cytokines were tested and found to be negative for MU-1 binding: mIL-2, hIL-2, hIL-15, mIL-7, TSLP, TSLP+IL7, TSLP+IL7R, TSLP+IL7g, TSLP+ IL-2, TSLP + IL2 + IL2Rbeta, IL2Rbeta, IL2Rgamma, IL7R, IL2+2Rbeta, IL2+2Rgamma, IL15+IL2Rbeta, IL15+2Rgamma, IL7+2Rgamma, IL2+IL7R,
- 5 IL15+IL7R, IL7+IL7R. Known receptors have been immobilized as well and tested for MUFc binding with negative results. IL-15 will bind to IL2Rb but not IL2Rg or MUFc.

EQUIVALENTS

- Those skilled in the art will recognize, or be able to ascertain using no more than
- 10 routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - 5 (a) the nucleotide sequence of SEQ ID NO:9;
 - (b) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code;
 - (c) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a);
 - 10 (d) a nucleotide sequence encoding a species homologue of the sequence of SEQ ID NO:10; and
 - (e) an allelic variant of the nucleotide sequence specified in (a).
2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes
15 for a protein having an MU-1 biological activity.
3. The polynucleotide of claim 2, wherein said biological activity is interaction with a STAT molecule.
- 20 4. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.
5. The polynucleotide of claim 1 comprising the nucleotide sequence of
SEQ ID NO:9.
25
6. A host cell transformed with the polynucleotide of claim 4.
7. The host cell of claim 6, wherein said cell is a mammalian cell.
- 30 8. A process for producing a MU-1 protein, said process comprising:

- (a) growing a culture of the host cell of claim 6 in a suitable culture medium;
and
- (b) purifying the MU-1 protein from the culture.

5 9. An isolated MU-1 protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10; and
- (b) fragments of (a) having an MU-1 biological activity.

10 10. The protein of claim 9 comprising the amino acid sequence of SEQ ID NO:10.

11. A pharmaceutical composition comprising a protein of claim 9 and a pharmaceutically acceptable carrier.

15

12. A protein produced according to the process of claim 8.

13. A composition comprising an antibody which specifically reacts with a protein of claim 9.

20

14. An isolated polynucleotide comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10; and
- 25 (b) fragments of (a) having an MU-1 biological activity.

15. The protein of claim 9 wherein said amino acid sequence is part of a fusion protein.

30 16. The protein of claim 15 comprising an Fc fragment.

17. The polynucleotide of claim 14 which encodes a peptide or protein comprising the amino acid sequence of SEQ ID NO:10.

18. An isolated polynucleotide comprising the nucleotide sequence of SEQ
5 ID NO:9, wherein said isolated polynucleotide encodes a MU-1 protein which interacts with a STAT molecule.

19. A method for modulating a STAT signaling pathway comprising
contacting a cell involved in STAT signaling with an agent capable of modulating MU-1
10 activity, thereby modulating a STAT signaling pathway.

20. The method of claim 19, wherein the STAT signaling pathway is a STAT
3 signaling pathway or a STAT 5 signaling pathway.

15 21. A method for identifying a compound capable of treating an immune disorder comprising assaying the ability of the compound to modulate MU-1 polypeptide interaction with a STAT molecule, thereby identifying a compound capable of treating an immune disorder.

20 22. The method of claim 21, wherein the STAT signaling pathway is a STAT 3 signaling pathway or a STAT 5 signaling pathway.

1/10

1 GTCGACGCGG CGGTACCAGC TGTCTGCCCA CTTCTCCTGT GGTGTGCCTC
51 ACGGTCACCTT GCTTGTCTGA CCGCAAGTCT GCCCATCCCT GGGGCAGCCA
101 ACTGGCCTCA GCCCGTGCCC CAGGCGTGCC CTGTCTCTGT CTGGCTGCCC
151 CAGCCCTACT GTCTTCCTCT GTGTAGGCTC TGCCCAGATG CCCGGCTGGT
201 CCTCAGCCTC AGGACTATCT CAGCAGTGAC TCCCCTGATT CTGGACTTGC
251 ACCTGACTGA ACTCCTGCCC ACCTCAAACC TTCACCTCCC ACCACCACCA
301 CTCCGAGTCC CGCTGTGACT CCCACGCCCA GGAGACCACC CAAGTGCCCC
351 AGCCTAAAGA ATGGCTTTCT GAGAAAGACC CTGAAGGAGT AGGTCTGGGA
401 CACAGCATGC CCCGGGGGCC AGTGGCTGCC TTACTCCTGC TGATTCTCCA
451 TGGAGCTTGG AGCTGCCTGG ACCTCACTTG CTACACTGAC TACCTCTGGA
501 CCATCACCTG TGTCTGGAG ACACGGAGCC CCAACCCAG CATACTCAGT
551 CTCACCTGGC AAGATGAATA TGAGGAACTT CAGGACCAAG AGACCTTCTG
601 CAGCCTACAC AGGTCTGGCC ACAACACCAC ACATATATGG TACACGTGCC
651 ATATGCGCTT GTCTCAATTC CTGTCCGATG AAGTTTTTCAT TGTCAATGTG
701 ACGGACCAGT CTGGCAACAA CTCCAAGAG TGTGGCAGCT TTGTCCTGGC
751 TGAGAGCATC AAACCAGCTC CCCCCTTGAA CGTGACTGTG GCCTTCTCAG
801 GACGCTATGA TATCTCCTGG GACTCAGCTT ATGACGAACC CTCCA ACTAC
851 GTGCTGAGGG GCAAGCTACA ATATGAGCTG CAGTATCGGA ACCTCAGAGA
901 CCCCTATGCT GTGAGGCCGG TGACCAAGCT GATCTCAGTG GACTCAAGAA
951 ACGTCTCTCT TCTCCCTGAA GAGTTCCACA AAGATTCTAG CTACCAGCTG
1001 CAGGTGCGGG CAGCGCCTCA GCCAGGCACT TCATT CAGGG GGACCTGGAG
1051 TGAGTGGAGT GACCCCGTCA TCTTTCAGAC CCAGGCTGGG GAGCCCGAGG
1101 CAGGCTGGGA CCCTCACATG CTGCTGCTCC TGGCTGTCTT GATCATTGTC
1151 CTGGTTTTCA TGGGTCTGAA GATCCACCTG CCTTGGAGGC TATGGAAAAA
1201 GATATGGGCA CCAGTGCCCA CCCCTGAGAG TTTCTTCCAG CCCCTGTACA
1251 GGGAGCACAG CGGGAACCTC AAGAAATGGG TTAATACCCC TTTCACGGCC
1301 TCCAGCATAG AGTTGGTGCC ACAGAGTTCC ACAACAACAT CAGCCTTACA
1351 TCTGTCAATTG TATCCAGCCA AGGAGAAGAA GTTCCCGGG CTGCCGGGTC
1401 TGGAAGAGCA ACTGGAGTGT GATGGAATGT CTGAGCCTGG TCACTGGTGC

Fig. 1

2/10

1451 ATAATCCCCT TGGCAGCTGG CCAAGCGGTC TCAGCCTACA GTGAGGAGAG
1501 AGACCGGCCA TATGGTCTGG TGTCCATTGA CACAGTGACT GTGGGAGATG
1551 CAGAGGGCCT GTGTGTCTGG CCCTGTAGCT GTGAGGATGA TGGCTATCCA
1601 GCCATGAACC TGGATGCTGG CCGAGAGTCT GGCCCTAATT CAGAGGATCT
1651 GCTCTTGGTC ACAGACCCTG CTTTTCTGTC TTGCGGCTGT GTCTCAGGTA
1701 GTGGTCTCAG GCTTGGAGGC TCCCCAGGCA GCCTACTGGA CAGGTTGAGG
1751 CTGTCATTTG CAAAGGAAGG GGACTGGACA GCAGACCCAA CCTGGAGAAC
1801 TGGGTCCCCA GGAGGGGGCT CTGAGAGTGA AGCAGGTTCC CCCCTGGTC
1851 TGGACATGGA CACATTTGAC AGTGGCTTTG CAGGTTCAGA CTGTGGCAGC
1901 CCCGTGGAGA CTGATGAAGG ACCCCCTCGA AGCTATCTCC GCCAGTGGGT
1951 GGTCAGGACC CCTCCACCTG TGGACAGTGG AGCCCAGAGC AGCTAGCATA
2001 TAATAACCAG CTATAGTGAG AAGAGGCCTC TGAGCCTGGC ATTTACAGTG
2051 TGAACATGTA GGGGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG
2101 TGTGTGTGTG TGTGTGTGTG TGTCTTGGGT TGTGTGTTAG CACATCCATG
2151 TTGGGATTTG GTCTGTTGCT ATGTATTGTA ATGCTAAATT CTCTACCCAA
2201 AGTTCTAGGC CTACGAGTGA ATTCTCATGT TTACAAACTT GCTGTGTAAA
2251 CCTTGTTCTT TAATTTAATA CCATTGGTTA AATAAAATTG GCTGCAACCA
2301 ATTACTGGAG GGATTAGAGG TAGGGGGCTT TTGAGTTACC TGTTTGGAGA
2351 TGGAGAAGGA GAGAGGAGAG ACCAAGAGGA GAAGGAGGAA GGAGAGGAGA
2401 GGAGAGGAGA GGAGAGGAGA GGAGAGGAGA GGAGAGGAGA GGAGAGGAGA
2451 GGCTGCCGTG AGGGGAGAGG GACCATGAGC CTGTGGCCAG GAGAAACAGC
2501 AAGTATCTGG GGTACACTGG TGAGGAGGTG GCCAGGCCAG CAGTTAGAAG
2551 AGTAGATTAG GGGTGACCTC CAGTATTTGT CAAAGCCAAT TAAAATAACA
2601 AAAAAAAAAA AAAAGCGGCC GCTCTAGA

Fig. 1 Cont.

3/10

1 MPRGPVAALL LLILHGAWSC LDLTCTYTDYL WTITCVLETR SPNPSILSLT
51 WQDEYEELQD QETFCSLHRS GHNTTHIWYT CHMRLSQFLS DEVFIVNVT
101 QSGNNSQECG SFVLAESIKP APPLNVTVAF SGRYDISWDS AYDEPSNYVL
151 RGKLQYELQY RNLRDYPYAVR PVTKLISVDS RNVSLLPEEF HKDSSYQLQV
201 RAAPQPGTSF RGT**WSEWS**DP VIFQTQAGEP EAGWDPHMLL LLAVLIIVLV
251 FMGLKIHLPW RLWKK**IWAPV** PTPESFFQPL YREHSGNFKK WVNTPTFTASS
301 IELVPQSSTT TSALHLSLYP AKEKKFPGLP GLEEQLECDG MSEP GHWCII
351 PLAAGQAVSA YSEERDRPYG LVSIDTVTVG DAEGLCVWPC SCEDDGYPAM
401 NLDAGRESGP NSEDLLLVT D PAFLSCGCVS GSGLRLGGSP GSLLDRLRLS
451 FAKEGDWTAD PTWRTGSPGG GSESEAGSPP GLDMDTFDSG FAGSDCGSPV
501 ETDEGPFRSY LRQWVVRTPP PVDGAQSS

Fig. 2

4/10

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huMU-1  .....NNGTCTGACTGGAGGCCCAGCTGCCCGTCATCA 32
murMU-1  CAGCCCTACTGTCTTCCTCTGTGTAGGCTCTGCCCAGATGCCCGGC.... 196
huMU-1  GAGTGACAGGTCTTATGACAGCCTGATTGGTGACTCGGGCTGGGTGTGGA 82
murMU-1  TGGTCCTCAGCCTCAGGACTATCTCAGCAGTGACTC.CCCTGATTCTGGA 245
huMU-1  TTCTCACCCAGGCCTCTGCCTGCTTTCTCAGACCCCTCATCT...GTCAC 129
murMU-1  CTTGCACCTGACTGAACTCCTGCCACCTCAAACCTTCACCTCCCACCAC 295
huMU-1  CCCACGCTGAACCCAGCTG.....CCACCCCCAGAAGCCCATCAGACT 173
murMU-1  CACCACTCCGAGTCCCGCTGTGACTCCACGCCCAGGAGACCACCCAAGT 345
huMU-1  GCCCCCAGCACACGGAATGGATTTCTGAGAAAGAAGCCGAAACAGAAGGC 223
murMU-1  G.CCCCAGCCTAAAGAATGGCTTTCTGAGAAAGACCCTGAAGGAGTAGGT 394
huMU-1  CCGTGAGGAGTCAGCATGCCGCGTGCTGGGCGCCCCCTTGCTCCTGCTG 273
murMU-1  C..TGGGACACAGCATGCCCGGGGCCAGTGGCTGCCTTACTCCTGCTG 442
huMU-1  CTGCTCCAGGGAGGCTGGGGCTGCCCCGACCTCGTCTGCTACACCGATT 323
murMU-1  ATTCTCCATGGAGCTTGGAGCTGCCTGGACCTCACTTGCTACACTGACT 492
huMU-1  CCTCCAGACGGTCATCTGCATCCTGGAAATGTGGAACCTCCACCCAGCA 373
murMU-1  CCTCTGGACCATCACCTGTGTCTGGAGACACGGAGCCCCAACCCAGCA 542
huMU-1  CGCTCACCCCTTACCTGGCAAGACCAGTATGAAGAGCTGAAGGACGAGGCC 423
murMU-1  TACTCAGTCTCACCTGGCAAGATGAATATGAGGAACTTCAGGACCAAGAG 592
huMU-1  ACCTCCTGCAGCCTCCACAGGTCTGGCCCAATGCCACGCATGCCACCT 473
murMU-1  ACCTTCTGCAGCCTACACAGGTCTGGCCACAACACCACACATATATGGTA 642
huMU-1  CACCTGCCACATGGATGTATTCCACTTCATGGCCGACGACATTTTCAGTG 523
murMU-1  CACGTGCCATATGCGCTTGTCTCAATTCCTGTCCGATGAAGTTTTCATTG 692
huMU-1  TCAACATCACAGACCAGTCTGGCAACTACTCCCAGGAGTGTGGCAGCTTT 573
murMU-1  TCAATGTGACGGACCAGTCTGGCAACAACCTCCAAGAGTGTGGCAGCTTT 742
huMU-1  CTCCTGGCTGAGAGCATCAAGCCGGCTCCCCCTTTCAACGTGACTGTGAC 623
murMU-1  GTCCTGGCTGAGAGCATCAAACCAGCTCCCCCTTGAACGTGACTGTGGC 792
huMU-1  CTTCTCAGGACAGTATAATATCTCCTGGCGCTCAGATTACGAAGACCCTG 673
murMU-1  CTTCTCAGGACGCTATGATATCTCCTGGGACTCAGCTTATGACGAACCCT 842
huMU-1  CCTTCTACATGCTGAAGGGCAAGCTTCAGTATGAGCTGCAGTACAGGAAC 723
murMU-1  CCAACTACGTGCTGAGGGGCAAGCTACAATATGAGCTGCAGTATCGGAAC 892

```

Fig. 3

5/10

huMU-1	CGGGGAGACCCCTGGGCTGTGAGTCCGAGGAGAAAGCTGATCTCAGTGG	773
murMU-1	CTCAGAGACCCCTATGCTGTGAGGCCGGTGACCAAGCTGATCTCAGTGG	942
huMU-1	CTCAAGAAGTGTCTCCCTCCTCCCCCTGGAGTTCCGCAAAGACTCGAGCT	823
murMU-1	CTCAAGAAACGTCTCTCTTCTCCCTGAAGAGTTCCACAAAGATTCTAGCT	992
huMU-1	ATGAGCTGCAGGTGCGGGCAGGGCCCATGCCTGGCTCCTCCTACCAGGGG	873
murMU-1	ACCAGCTGCAGGTGCGGGCAGCGCCTCAGCCAGGCACTTCATTTCAGGGGG	1042
huMU-1	ACCTGGAGTGAATGGAGTGACCCGGTCATCTTTTCAGACCCAGTCAGAGGA	923
murMU-1	ACCTGGAGTGAGTGGAGTGACCCCGTCATCTTTTCAGACCCAGGCTGGGGA	1092
huMU-1	GTTAAAGGAAGGCTGGAACCTCACCTGCTGCTTCTCCTCCTGCTTGTCA	973
murMU-1	GCCCGAGGCAGGCTGGGACCCTCACATGCTG...CTGCTCCTGGCTGTCT	1139
huMU-1	TAGTCTTCATTCCTGCCTTCTGGAGCCTGAAGACCCATCCATTGTGGAGG	1023
murMU-1	TGATCATTTGTCTGTTTTCATGGGTCTGAAGATCCACCTGCCTTGGAGG	1189
huMU-1	CTATGGAAGAAGATATGGG...CCGTCCCCAGCCCTGAGCGGTTCTTCAT	1070
murMU-1	CTATGGAAAAAGATATGGGCACCAGTGCCACCCCTGAGAGTTTCTTCCA	1239
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murMU-1	GCCCCGTGTACAGGGAGCACAGCGGGAACCTTCAAGAAATGGGTAAATACCC	1289
huMU-1	CCTTCACTGGCTCCAGCCTGGAGCTGGGACCCTGGAGCCCAGAGGTGCCC	1170
murMU-1	CTTTCACGGCCTCCAGCATAGAGTTGGTGCCACAGAGTTCCACAACAACA	1339
huMU-1	TCCACCCTGGAGGTGTACAGCTGCCACCCACCACGGAGCCCGGCCAAGAG	1220
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murMU-1	GAAGAAGTTCCCGGGGCTGCCGGGTCTGGAAGAGCAACTGGAGTGTGATG	1424
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murMU-1	GAATGCTGTAGCCTGGTCACTGGTGCATAATCCCCTTGGCAGCTGGCCAA	1474
huMU-1	GGGGGCTCAGCTTACAGTGAGGAGAGGGATCGGCCATACGGCCTGGTGTCT	1361
murMU-1	GCGGTCTCAGCCTACAGTGAGGAGAGAGACCGGCCATATGGTCTGGTGTCT	1524
huMU-1	CATTGACACAGTGAAGTGTGCTAGATGCAGAGGGGCCATGCACCTGGCCCT	1411
murMU-1	CATTGACACAGTGAAGTGTGGGAGATGCAGAGGGCCTGTGTGTCTGGCCCT	1574
huMU-1	GCAGCTGTGAGGATGACGGCTACCCAGCCCTGGACCTGGATGCTGGCCTG	1461
murMU-1	GTAGCTGTGAGGATGATGGCTATCCAGCCATGAACCTGGATGCTGGCCGA	1624
huMU-1	GAGCCCAGCCAGGCCTAGAGGACCCACTCTTGGATGCAGGGACCACAGT	1511
murMU-1	GAGTCTGGCCCTAATTCAGAGGATCTGCTCTTGGTCACAGACCCTGCTTT	1674

Fig. 3 Cont.

6/10

huMU-1	CCTGTCCTGTGGCTGTGTCTCAGCTGGCAGCCCTGGGCTAGGAGGGCCCC	1561
murMU-1	TCTGTCTTGCGGCTGTGTCTCAGGTAGTGGTCTCAGGCTTGGAGGCTCCC	1724
huMU-1	TGGGAAGCCTCCTGGACAGACTAAAGCCAACCCCTTGCAGATGGGGAGGAC	1611
murMU-1	CAGGCAGCCTACTGGACAGGTTGAGGCTGTCAATTTGCAAAGGAAGGGGAC	1774
huMU-1	TGGGCTGGGGGACTGCCCTGGGGTGGCCGGTCACCTGGAGGGGTCTCAGA	1661
murMU-1	TGGACAGCAGACCCAACCTGGAGAACTGGGTCCCCAGGAGGGGGCTCTGA	1824
huMU-1	GAGTGAGGCGGGCTCACCCCTGGCCGGCCTGGATATGGACACGTTTGACA	1711
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murMU-1	CAGGACCCCTCCACCTGTGGACAGTGGAGCCCAGAGCAGCTA.....	1995
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murMU-1	TGTCTT.GGGTTGTGT	2135
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huMU-1	GGCGGAGCCCAGCCCTCCAGCGTCTGCCTCCAGGAGCTGCAAGAAGTCCA	2261
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huMU-1	TATTG...TTCCTTATCACCCTGCCAACAGGAAGCGAAAGGGGATGGAG	2306
murMU-1	GGTAGGGGGCTTTTGAGTTACCTGTTTGGAGATGGAGAAGGAGAGAGGAG	2368

Fig. 3 Cont.

huMU-1	TGAGCCCATGGTGACCTCGGGAATGGCAATTTT'TGGGCGGCCCTGGAC	2356
murMU-1	AGACCAAGAGGAGAAGGAGGAAGGAGAGGAGAGGAGAGGAGAGGAGAGGA	2418
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murMU-1	AAAAAAAAAAGCGGCCGCTCTAGA	2628

SUBSTITUTE SHEET (RULE 26)

8/10

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MurineMU-1	QSGNNSQECGSFVLAESIKPAPPLNVTVAFSGRYDISWDSAYDEPSNYVL	150
Human MU-1	KGKLQYELQYRNRGDPWAVSPRRKLISVDSRSVSLPLEFRKDSSYELQV	200
MurineMU-1	RGKLQYELQYRNLRDPYAVRPVTKLISVDSRNVSLPPEEFHKDSSYQLQV	200
Human MU-1	RAGPMPGSSYQGTWSEWSDPVIFQTQSEELKEGWNPHLLLLLLLLLVIVFIP	250
MurineMU-1	RAAPQPGTSFRGTWSEWSDPVIFQTQAGEPEAGWDPHMLLLLAVALIIVL	249
Human MU-1	AFWSLKTHTPLWRLWKKIWA.VSPSPERFFMPPLYKGCSDGDFKKWVGAPFTGS	299
MurineMU-1	VFMGLKIHLPWRLWKKIWAPVPTPESFFQPLYREHSGNFKKWVNTPTFTAS	299
Human MU-1	SLELGPWSPEVPSTLEVYSCHPPRSPAKRLQLTELQEPALVESDGVPKP	349
MurineMU-1	SIELVPQSSTTTTAL.....HLSLYPAKEKKFPGLPGLLEEQLCECDGMSEP	344
Human MU-1	SFW...PTAQNSGGSAYSEERDRPYGLVSIIDTVTVLDAEGPCTWPCSCED	396
MurineMU-1	GHWCIIPLAAGQAVSAYSEERDRPYGLVSIIDTVTVGDAEGLCVWPCSCED	394
Human MU-1	DGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSL	446
MurineMU-1	DGYPAMNLDAGRESGPNSDLLLLVTDPAFLSCGCVSGSLRLGGSPGSL	444
Human MU-1	DRLKPPLADGEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTFDSGFVG	496
MurineMU-1	DRLRLSFAKEGDWTADPTWRTGSPGGGSESEAGSP.PGLDMDTFDSGFAG	493
Human MU-1	SDCSSPVECDFTSPGDEGPPRSYLRQWVV.IPPPLSSPGPQAS*	539
MurineMU-1	SDCGSPVET.....DEGPPRSYLRQWVVRTPPFVDS.GAQSS.	529

Fig. 4

9/10

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mousemu	~~~MPRGPVA	ALLLLI..LH	GAWG..... CLDLTCYTDY LWTITCVLET
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humu	WN..LHPSTL	TLTWQDQYEE	LKDEATSCSL HRSAHNATHA TYTCHM....
mousemu	RS..PNPSIL	SLTWQDEYEE	LQDQETFCSL HRSGHNTTHI WYTCHM....
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humu	..SGQYNISW	RSDYEDPAFY	MLKGKLQYEL QYRNRGDPWA VSPRRKLISV
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mousemu	DSRNVSLPE	EFHKDSSYQL	QVRAAPQPGT SFRGTWSEWS DPVIFQTA.
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humu	..EELKEGWN	PHLLLLL... LLVIVFIPAF	WSLKTHPLWR LWK KIWA.VP
mousemu	..GEPEAGWD	PHMLLLL... AVLIIVL.VF	MGLKIHLPLWR LWK KIWAPVP
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mousemu	DPTWRTGSPG	GGSESEAGSP	.PGLDMDTFD SGFAGSDCGS PVET.....
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humu	DEGPPRS Y LR	QWVVIPPLS	SPGPQAS*~~ ~~~~~
mousemu	DEGPPRS Y LR	QWVVRTPPV	DSGAQSS~~~ ~~~~~
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Fig. 5

10/10

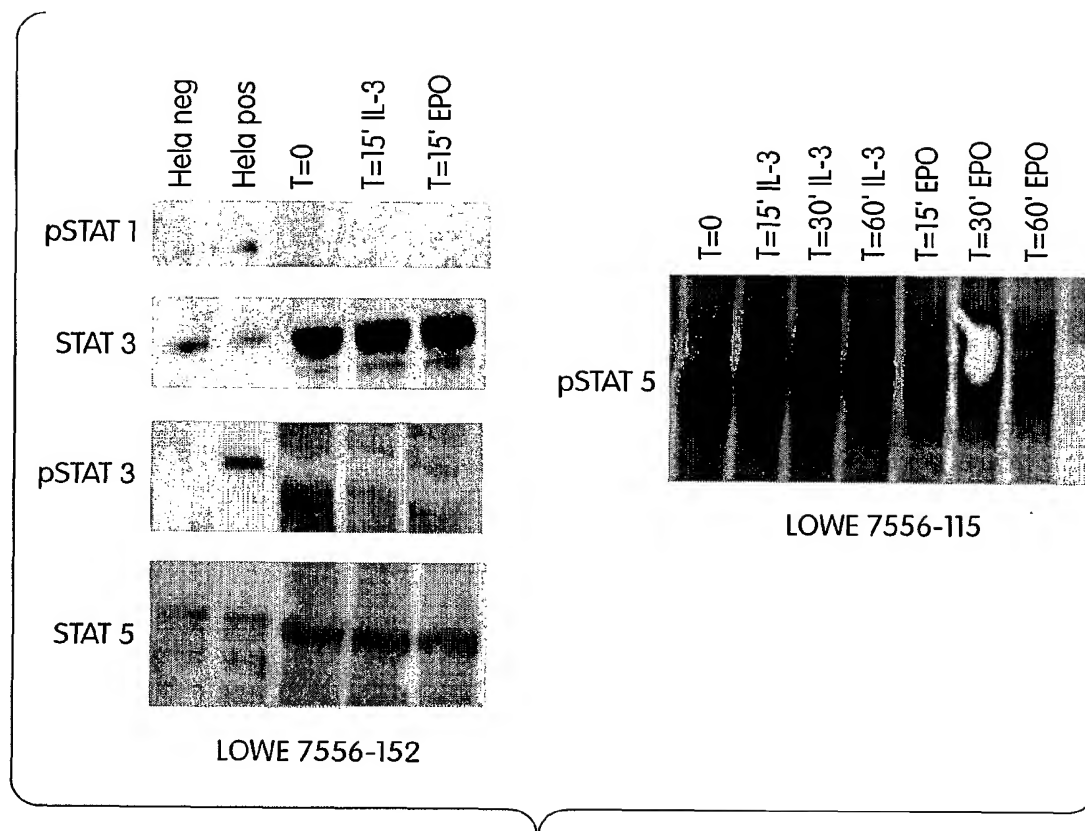


Fig. 6

SEQUENCE LISTING

<110> Genetics Institute, Inc., et al.

<120> MU-1, MEMBER OF THE CYTOKINE RECEPTOR FAMILY

<130> GFN-5230CPPC

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<151> 2000-05-11

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Leu Trp Ser Glu Trp Ser Gln Pro Ile Tyr Val Gly Asn Asp Glu His
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Phe Ile Leu Leu Ile Leu
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<212> PRT

<213> Homo sapiens

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 <212> PRT
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<400> 10
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 Ile Thr Cys Val Leu Glu Thr Arg Ser Pro Asn Pro Ser Ile Leu Ser
 35 40 45
 Leu Thr Trp Gln Asp Glu Tyr Glu Glu Leu Gln Asp Gln Glu Thr Phe
 50 55 60
 Cys Ser Leu His Arg Ser Gly His Asn Thr Thr His Ile Trp Tyr Thr
 65 70 75 80
 Cys His Met Arg Leu Ser Gln Phe Leu Ser Asp Glu Val Phe Ile Val
 85 90 95
 Asn Val Thr Asp Gln Ser Gly Asn Asn Ser Gln Glu Cys Gly Ser Phe
 100 105 110
 Val Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Leu Asn Val Thr Val
 115 120 125
 Ala Phe Ser Gly Arg Tyr Asp Ile Ser Trp Asp Ser Ala Tyr Asp Glu
 130 135 140
 Pro Ser Asn Tyr Val Leu Arg Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
 145 150 155 160
 Arg Asn Leu Arg Asp Pro Tyr Ala Val Arg Pro Val Thr Lys Leu Ile
 165 170 175
 Ser Val Asp Ser Arg Asn Val Ser Leu Leu Pro Glu Glu Phe His Lys
 180 185 190
 Asp Ser Ser Tyr Gln Leu Gln Val Arg Ala Ala Pro Gln Pro Gly Thr
 195 200 205
 Ser Phe Arg Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
 210 215 220
 Thr Gln Ala Gly Glu Pro Glu Ala Gly Trp Asp Pro His Met Leu Leu
 225 230 235 240
 Leu Leu Ala Val Leu Ile Ile Val Leu Val Phe Met Gly Leu Lys Ile
 245 250 255
 His Leu Pro Trp Arg Leu Trp Lys Lys Ile Trp Ala Pro Val Pro Thr
 260 265 270
 Pro Glu Ser Phe Phe Gln Pro Leu Tyr Arg Glu His Ser Gly Asn Phe
 275 280 285
 Lys Lys Trp Val Asn Thr Pro Phe Thr Ala Ser Ser Ile Glu Leu Val
 290 295 300
 Pro Gln Ser Ser Thr Thr Thr Ser Ala Leu His Leu Ser Leu Tyr Pro
 305 310 315 320
 Ala Lys Glu Lys Lys Phe Pro Gly Leu Pro Gly Leu Glu Glu Gln Leu
 325 330 335

Glu Cys Asp Gly Met Ser Glu Pro Gly His Trp Cys Ile Ile Pro Leu
 340 345 350
 Ala Ala Gly Gln Ala Val Ser Ala Tyr Ser Glu Glu Arg Asp Arg Pro
 355 360 365
 Tyr Gly Leu Val Ser Ile Asp Thr Val Thr Val Gly Asp Ala Glu Gly
 370 375 380
 Leu Cys Val Trp Pro Cys Ser Cys Glu Asp Asp Gly Tyr Pro Ala Met
 385 390 395 400
 Asn Leu Asp Ala Gly Arg Glu Ser Gly Pro Asn Ser Glu Asp Leu Leu
 405 410 415
 Leu Val Thr Asp Pro Ala Phe Leu Ser Cys Gly Cys Val Ser Gly Ser
 420 425 430
 Gly Leu Arg Leu Gly Gly Ser Pro Gly Ser Leu Leu Asp Arg Leu Arg
 435 440 445
 Leu Ser Phe Ala Lys Glu Gly Asp Trp Thr Ala Asp Pro Thr Trp Arg
 450 455 460
 Thr Gly Ser Pro Gly Gly Gly Ser Glu Ser Glu Ala Gly Ser Pro Pro
 465 470 475 480
 Gly Leu Asp Met Asp Thr Phe Asp Ser Gly Phe Ala Gly Ser Asp Cys
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<400> 13
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<400> 14
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<210> 16
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<212> DNA
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<400> 16
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Debra D. Donaldson, et al. -1-
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